JC18 Rec'd PCT/PTO 02 MAY 2001

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER: BO 41745 JGD/SMO U.S. APPLN NO. (if known, see 37 CFR 1.5) 09/830871
INTERNATIONAL APPLICATION NO.: PCT/NL99/00673	INTERNATIONAL FILING DATE: 2 November 1999 (02.11.99)	PRIORITY DATE CLAIMED: 2 November 1998 (02.11.98)
TITLE OF INVENTION: CARBOHYDRATE OXIDATION PRODUCTS AND DERIVATIVES		
APPLICANT(S) FOR DO/EO/US: Arie Cornelis BESEMER, Jan Matthijs JETTEN, Hendrik Arend VAN DOREN and Jan Pieter VAN DER LUGT		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [X] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   - a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [ ] have been transmitted by the International Bureau.
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: International Preliminary Examination Report (PCT/IPEA/409), Application Data Sheet

U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/830871	INTERNATIONAL APPLICATION NO PCT/NL99/00673	ATTORNEY'S DOCKET NO. BO 41745 JGD/SMO

CALCULATIONS PTO USE ONLY

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):
Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... $ 1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... $ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... $ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... $ 690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .... $ 100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$	860.00
Surcharge of $130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	130.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	15 - 20 =	0	X $18.00	$	
Independent claims	3 - 3 =	0	X $80.00	$	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$	990.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +				$	
SUBTOTAL =				$	990 00
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.49(f)).				$	
TOTAL NATIONAL FEE =				$	990.00
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$	990 00
				Amount to be refunded:	
				charged:	

a. [X] A check in the amount of $ **990** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

YOUNG & THOMPSON
745 South 23rd Street
2nd Floor
Arlington, VA 22202
(703) 521-2297
Facsimile (703) 685-0573
Customer Number: 000466

May 2, 2001

By Benoît Castel
Benoît Castel
Attorney for Applicant
Registration No. 35,041

09/830871

JC18 Rec'd PCT/PTO 02 MAY 2001

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Arie BESEMER et al.

Serial No. (unknown)

Filed herewith

CARBOHYDRATE OXIDATION PRODUCTS AND DERIVATIVES

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to the first Official Action and calculation of the filing fee, please substitute Claims 10-14 as originally filed, which appear on page 10, with Claims 10-14 as filed in the Article 34 amendment of December 5,2000. The page containing Claims 10-14 is marked "AMENDED SHEET" and is attached hereto. Following the insertion of Claims 10-14, please amend these claims as follows:

IN THE CLAIMS:

Cancel claims 1-14.

Add new claims 15-29.

--15.(New) An oxidized carbohydrate derived from a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, the 1,2-dihydroxyethylene groups having at least partially been oxidized to dialdehyde groups, and a part of the aldehyde groups having been oxidized to carboxylic acid groups, the ratio

between aldehyde groups and carboxyl groups being between *25/75* and 80/20.--

--16.(New) An oxidized carbohydrate according to claim 15, containing on average 0.1-1.5 carboxyl groups and *0.5-1.9* aldehyde groups per oxidized 1,2-dihydroxyethylene group.--

--17.(New) An oxidized carbohydrate according to claim 16, containing on average *0.5-1.3* carboxyl groups and 0.7-1.5 aldehyde groups per oxidized 1,2-dihydroxyethylene group.--

--18.(New) An oxidized carbohydrate according to claim 15, containing on average 0.1-1.2 carboxyl groups and 0.3-1.2 aldehyde groups per repeating unit.--

--19.(New) An oxidized carbohydrate according to claim 15, wherein the carbohydrate is selected from starch, amylose, amylopectin and modifications thereof.--

--20.(New) An oxidized carbohydrate according to claim 15, wherein the carbohydrate is selected from cellulose and modifications thereof.--

--21.(New) An oxidized carbohydrate according to claim 15, wherein the carbohydrate is a 2,1-fructan.--

--22.(New) A process for producing an oxidized carbohydrate containing aldehyde groups and carboxylic acid groups, the ratio between aldehyde groups and

carboxyl groups being between *25/75* and 80/20, the process comprising oxidizing a dialdehyde carbohydrate obtainable by oxidizing a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, the oxidation of the dialdehyde carbohydrate being performed with a catalytic amount of molecular halogen.--

--23.(New) A process according to claim 22, wherein the oxidation with molecular halogen is performed at a pH between 3 and 7.--

--24.(New) A process according to claim 22, wherein the molecular halogen is produced in situ by reaction of halide with a carboxylic peracid.--

--25.(New) A process according to claim 22, wherein the molecular halogen is molecular bromine.--

--26.(New) A process for producing an oxidized, amino-substituted carbohydrate, comprising reductively aminating residual aldehyde groups in the oxidized

carbohydrate obtained by the process according to claim 22.--

--27. (New) An amino-substituted oxidation product derived from a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, these dihydroxyethylene groups having at least partially been oxidized to dialdeltyde groups, the product containing on average 0.1-1.5 carboxyl groups and 0.1-1.9 substituted amine groups per oxidized 1,2-dihydroxyethylene group.--

--28. (New) An amino-substituted oxidation product according to claim 273, containing on average 0.1-1.2 carboxyl groups and 0.3-1.2 substituted amino groups per repeating unit.--

--29. (New) An amino-substituted oxidation product according to claim 27, wherein said substituted amino group has the formula $-NR^1R^2$, wherein R^1 represents hydrogen, a C_1C_{20} alkyl, alkenyl or alkynyl group optionally substituted with carboxy, hydroxy, C_1C_{12} alkoxy,

amino, carbamoyl and/or aryl, including natural and synthetic amino acid residues, and R^2 represents hydrogen, amino, substituted amino, hydroxy, alkoxy, or a C_1-C_{12} alkyl, ailcenyl or alkynyl group optionally substituted with carboxy, hydroxy, C_1-C_{12} alkoxy, amino and/or carbamoyl, or a substituted iminomethyl group, or R^1 and R^2, together with the nitrogen atom to which they are bound, represent a three- to seven- membered heterocyclic system, optionally containing one or more further heteroatoms selected from nitrogen, oxygen and sulphur and optionally substituted with carboxy, hydroxy, oxo, C_1-C_{12} alkyl, alkenyl, alkynyl or alkoxy, amino, carbamoyl and/or aryl.--

R E M A R K S

The above changes in the claims merely place this national phase application in the same condition as it was during Chapter II of the international phase, with the

multiple dependencies being removed. Following entry of this amendment by substitution of the pages, only claims 15-29 remain pending in this application.

Respectfully submitted,

YOUNG & THOMPSON

By Benoît Castel

Benoît Castel
Attorney for Applicant
Customer No. 000466
Registration No. 35,041
745 South 23rd Street
Arlington, VA 22202
703/521-2297

May 2, 2001

10. A process according to claim 7 or 8, wherein the molecular halogen is produced in situ by reaction of halide with a carboxylic peracid.

11. A process for producing an oxidised, amino-substituted carbohydrate, comprising reductively aminating residual aldehyde groups in the oxidised carbohydrate obtained by the process according to any one of claims 8-10.

12. An amino-substituted oxidation product derived from a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, these dihydroxyethylene groups having at least partially been oxidised to dialdehyde groups, the product containing on average 0.1-1.5 carboxyl groups and 0.1-1.9 substituted amine groups per oxidised 1,2-dihydroxyethylene group.

13 An amino-substituted oxidation product according to claim 12, containing on average 0.1-1.2 carboxyl groups and 0 3-1.2 substituted amino groups per repeating unit.

14. An amino-substituted oxidation product according to claim 12 or 13, wherein said substituted amino group has the formula $-NR^1R^2$, wherein R^1 represents hydrogen, a C_1-C_{20} alkyl, alkenyl or alkynyl group optionally substituted with carboxy, hydroxy, C_1-C_{12} alkoxy, amino, carbamoyl and/or aryl, including natural and synthetic amino acid residues, and R^2 represents hydrogen, amino, substituted amino, hydroxy, alkoxy, or a C_1-C_{12} alkyl, alkenyl or alkynyl group optionally substituted with carboxy, hydroxy, C_1-C_{12} alkoxy, amino and/or carbamoyl, or a substituted iminomethyl group, or R^1 and R^2, together with the nitrogen atom to which they are bound, represent a three- to seven-membered heterocyclic system, optionally containing one or more further heteroatoms selected from nitrogen, oxygen and sulphur and optionally substituted with carboxy, hydroxy, oxo, C_1-C_{12} alkyl, alkenyl, alkynyl or alkoxy, amino, carbamoyl and/or aryl

09/830871
JC18 Rec'd PCT/PTO 02 MAY 2001

CARBOHYDRATE OXIDATION PRODUCTS AND DERIVATIVES

The present invention relates to novel oxidation products of carbohydrates and derivatives thereof, and to processes of preparing these.

Several procedures are known in the art for subjecting chemical species containing 1,2-dihydroxyethylene units to 'oxidative glycol cleavage', a carbon-carbon scission reaction which is accompanied by oxidation of the hydroxymethine groups to aldehydes or carboxylic acids. In polymeric carbohydrates, in which the 1,2-dihydroxyethylene units are usually part of a 5- or 6-membered ring, the carbon-carbon bond scissions do not lead to degradation of the polymeric chains. The best-known procedures for said conversions in carbohydrates are:

- sodium periodate oxidation to dialdehydes (see e.g. WO 95/12619), which, if so desired, can be followed by oxidation to the corresponding diacids by reaction with sodium chlorite and hydrogen peroxide, and
- direct conversion of carbohydrates to diacids by reaction with sodium hypochlorite and a catalytic amount of bromine (see e.g. EP-A-427349).

In principle, such conversions can be carried out with any carbohydrate containing 1,2-dihydroxyethylene units, but most of the work known in the art has been restricted to dialdehyde starch and dialdehyde inulin, and dicarboxylic starch and dicarboxylic inulin, respectively. Dialdehyde derivatives of carbohydrates have been reported to be useful as additives in papermaking processes (wet-end strengthening); dicarboxylic derivatives of carbohydrates are useful due to their capacity to bind divalent metal ions, notably calcium and magnesium ions.

Another reaction of dialdehyde starch was described by Jeanes and Hudson, *J. Org. Chem.* **20**, 1565-1568 (1955). They heated the periodate-oxidised starch at 100°C for 45 minutes and then treated the cooled solution with barium acetate and 2 equivalents of bromine, ultimately resulting in the generation of erythritol and erythronic lactone from the periodate-oxidised starch. Under those heating conditions, the dialdehyde groups of the oxidised starch will disproportionate to carboxyl and alcohol groups by the well-known Cannizzaro reaction. Moreover, the process is accompanied by considerable chain degradation. Thus the reaction product is not a polymeric product having appreciable levels of aldehyde groups in addition to the carboxylic groups.

It was found now that in 'dialdehyde carbohydrates', i.e. carbohydrates in which the 1,2-dihydroxyethylene groups of the parent carbohydrate have been partly or completely converted to dialdehyde groups, one of the aldehyde groups can be selectively oxidised to a carboxyl group. The mono-carboxyl-monoaldehyde carbohydrate derivatives thus obtained have interesting properties and can be applied e.g. as temporary crosslinkers in polysaccharide solutions or suspensions or as reactive hydrophilic coatings. They are also versatile starting materials for further derivatisation, especially to amino derivatives, to obtain e.g. polymeric surfactants, emulsifiers or decoupling polymers. The carboxyl-aldehyde carbohydrates of the invention have an aldehyde to carboxyl ratio of between 25/75 and 80/20, especially between 40/60 and 75/25. They contain on average 0.1-1.5, preferably 0.5-1.3 carboxyl group, and 0.5-1.9, preferably 0.7-1.5 aldehyde group per oxidised 1,2-dihydroxyethylene group. Per total number of repeating units (including non-oxidised units if any), the products of the invention contain on average 0.1-1.2, preferably 0.2-1.0 carboxyl group and 0.2-1.5, preferably 0.3-1.2 aldehyde group per repeating unit.

The present invention also includes a new process for the oxidation of aldehyde groups to carboxylic groups in carbohydrate derivatives, in which only a catalytic amount of molecular halogen is required. The catalytic amount of halogen is regenerated in situ by oxidation with an oxidising agent. Preferably, this novel process comprises the use of peracids for the (re)generation of the molecular halogen instead of sodium hypochlorite. It was found that the novel process, besides reducing the amount of halide produced, is also beneficiary to the properties of the partially oxidised products, in that a lower extent of degradation is observed. In addition, this process is considerably cheaper than the oxidation with sodium chlorite in the presence of hydrogen peroxide.

The catalytic amount of molecular halogen may be 0.2-40, preferably from 1 to 10 mole%, with respect to the amount of peracid. The halogen may be chlorine, bromine or iodine. The peracid may be any peralkanoic acid such as peracetic acid, perpropionic acid, perlauric acid etc., a substituted peralkanoic acid such as peroxytrifluoroacetic acid, an optionally substituted aromatic peracid such as perbenzoic acid or m-chloroperbenzoic acid, or an inorganic peracid such as perboric or persulphuric acid.

The oxidised carbohydrate according to the invention can be derived from any carbohydrate containing 1,2-dihydroxyethylene groups in its recurring units, which carbohydrate contains a relatively low level of reducing end groups. Such carbohydrates include non-

reducing disaccharides and oligosaccharides, such as sucrose, raffinose, trehalose and similar oligosaccharides, and polysaccharides which are 1,2-, 1,4- or 1,6-linked. Examples include α-1,4-glucans (the "starch family"), β-1,4-glucans (cellulose), glucomannans and galactomannans (guar and locust bean gum), (arabino)xylans (hemicellulose) and β-2,1- and β-2,6-fructans (inulin and levan). The starch-type carbohydrates, cellulose and inulin are preferred carbohydrates.

Modifications of starch and other carbohydrates an also be used as starting materials, and comprise partially hydrolysed products, as well as physical and chemical modifications, including hydroxyalkyl, carboxyalkyl and similar derivatives, as well as uronic analogues. Short-chain carbohydrate derivatives, including monosaccharides, in which the reducing end groups have been protected, e.g. as glycosides, are also suitable starting materials. The carbohydrates are oxidised to dialdehyde derivatives by (meta)periodate oxidation or any other suitable method, such as methods using manganese oxides. The oxidation may be complete, i.e. the oxidised carbohydrate may exclusively consist of dialdehyde monose units, or the oxidation may be partial, i.e. to a degree of oxidation (dialdehyde monose units) of 0.1-0.99 or 0.2-0.8.

After (partial) oxidation of the 1,2-dihydroxyethylene groups in a carbohydrate to obtain the corresponding dialdehyde derivative, this product is further oxidised by reacting it with molecular halogen, preferably bromine, in the presence of an oxidising agent such as hypochlorite or a suitable peracid, preferably peracetic acid. The reaction can be performed in an aqueous slurry or solution, at a pH of 3-7, preferably 4-6. The reaction temperature is typically from 0 up to 80°C, preferably up to 50°C, more preferably from 0°C to ambient temperature. The reaction may be carried out in a closed system to avoid loss of halogen by evaporation. A product is obtained, in which 0.1-1.5, preferably 0.6-1.2, aldehyde function in each oxidised 1,2-dihydroxyethylene group has been converted to carboxylic acid groups. The carboxylic acid groups may be present in the product in the form of the free acids, their carboxylate salts (e.g. with metal or (substituted) ammonium ions), as 4-7 membered lactones, or as mixtures thereof. The remaining aldehyde groups may be present as such, in the form of their hydrates or as (hemi-)acetals or (hemi-)aldals.

Using the process of the invention, a distinct decrease in the reaction rate is observed when the degree of oxidation reaches about 50%, i.e. when about 50% of the available aldehyde groups have been converted to carboxylic groups. It is believed that the oxidation takes place in such a manner that one of the aldehyde functions in each monomeric unit reacts

first, whereas the oxidation of the other aldehyde function proceeds more slowly, most likely due to the formation of stable cyclic hemi-acetals. As a result, the reaction can be stopped conveniently at this stage, and a product is obtained in which approximately equal amounts of aldehyde and carboxylic functions are present.

The product has as a characteristic feature that essentially all dialdehyde functions have been converted to mono-aldehyde-monocarboxylic functions. In the case of amylose that has been converted fully to dialdehyde amylose, oxidation according to the process of the present invention will lead to a structurally regular product with alternating aldehyde and carboxylic groups. So far, it has been notoriously difficult to obtain structural regularity in carbohydrates derivatives. The products of the invention provide novel material properties.

The oxidised carbohydrates of the present invention can also serve as starting materials for a range of derivatives. The aldehyde is especially useful as an anchor for further derivatisation. The reaction of the residual aldehyde groups with amines is of particular interest and leads to products that are structurally quite different from reaction products of amines and dialdehyde functions. In the latter case, when primary amines are used, each amine group will usually react with both aldehyde groups in the dialdehyde moiety, supposedly leading to seven-membered rings incorporating one nitrogen atom (*see* Guthrie, *Advances in Carbohydrate Chemistry*, Vol. 16, 1961). In the case of the oxidised carbohydrates of the present invention, however, the remaining aldehyde function of each mono-aldehyde-mono-carboxylic moiety will react with an amine group, leading to an acyclic moiety. The resulting products have interesting properties, associated with their zwitter-ionic (both catonic and anionic) nature and surface-active (both polar and apolar parts) and metal-binding properties.

The amines that can be coupled to the mono-aldehyde-mono-acids include primary and secondary amines having the formula HNR^1R^2, with R^1 and R^2 as defined in the claims. Examples of amines are ammonia, alk(en)ylamines such as methyl, allyl, butyl, decyl, hexadecyl or octadecenyl, dialkyl amines, cyclic amines such as pyrrolidines, piperidines, morpholines, thiazolines, imidazoles, tetrazoles, triazines etc., carboxy-substituted amines such as glycine, lysine or other α-amino acids, or iminodiacetic acid, hydroxy-substituted amines such as diethanolamine, and diamines and polyamines such as hexamethylene-diamine, and further other amino-functional molecules that can react with aldehyde functions such as hydrazine, hydrazides, hydrazones, hydroxylamines, amidines, guanidines, isothiourea's and the like. The latter can be used for crosslinking the carbohydrates.

Coupling of the amines to the aldehyde function results primarily in imines, which are usually not thermally and hydrolytically stable, and are, therefore, preferably reduced to the corresponding amines using conventional reducing agents such as borohydrides. The reductive amination can also be performed in a single step, using reducing agents such as borohydrides or using pressurised hydrogen in the presence of a metal catalyst. These amination reactions as such are well-known to the skilled person.

The amino-substituted carbohydrate oxidation product of the invention may contain on average 0.1-1.5, preferably 0.3-1.3 carboxyl group and 0.1-1.9, preferably 0.3-1.5 substituted amine group per oxidised 1,2-dihydroxyethylene group. Per total number of repeating units (including non-oxidised units if any), they may contain on average 0.1-1.2, preferably 0.2-1.0 carboxyl group, and 0.1-1.5, preferably 0.3-1.2 substituted amino groups per repeating unit. In addition to the amino groups, the product may or may not contain residual carbonyl (aldehyde) groups; in other words, the amination of the aldehyde groups may be partial or complete.

Example 1

Preparation of a starch derivative containing approximately equivalent amounts of aldehyde and carboxylic functions (Monoaldehyde monocarboxylic starch, MACS)

1a. Preparation of dialdehyde starch (DAS)

122.5 grams (0.76 mole, based on anhydroglucose) of starch (weight corrected for dry matter content) are suspended in 500 ml of demineralised water. The suspension is brought to pH 4.5 and cooled to 5°C. Sodium periodate (165 g, 0.77 mole) is added and the suspension is stirred at 5°C in the dark for 20 hours. The dialdehyde starch obtained in this fashion is isolated by filtration. The crude product is washed extensively with water until iodate can no longer be detected by reaction with potassium iodide.

1b. Preparation of monoaldehyde monocarboxylic starch (MACS)

The DAS thus prepared was oxidised further using bromine/peracetic acid. In order to oxidise approximately 50% of the aldehyde groups present, 0.76 mole of peracetic acid (0.584 M solution, 1,3 l) was added in 14 portions to the well-stirred suspension of DAS in 1 l of water, to which 12 g (0.12 mole, corresponding to 0.06 mole of Br_2) of sodium bromide had been added. This amount was sufficient to effect complete conversion of peracetic acid to acetic acid through oxidation of bromide to bromine, which is the oxidising species in the reaction. The pH was kept at 5 (addition of 0.1 N sodium hydroxide solution, pH-stat) throughout the reaction and each consecutive portion of peracetic acid was added after the suspension/solution had turned colourless. Upon

completion of the reaction, the solution was desalinated by ultrafiltration, using a membrane with a cut-off MW 5000, and freeze-dried.

Characterisation of MACS

Carboxylic acid content: Desalinated MACS was dissolved in a small volume of demineralised water and eluted over a cation-exchange resin (H^+-form). The eluate was freeze-dried and titrated with sodium hydroxide solution. The carboxylic content was found to be about 0.7 carboxylic groups per monomer unit.

Aldehyde content: An excess of hydroxylammonium chloride was reacted with desalinated MACS. The hydrochloric acid that was liberated upon reaction with the aldehyde functions was titrated with sodium hydroxide solution. The aldehyde content was found to be about 1.2 aldehyde functions per monomer unit.

The ratio of aldehyde to carboxylic acid is therefore approximately 60-40.

Example 2

Reductive amination of MACS with L-aspartic acid

5.0 g of lyophilised MACS, prepared according to Example 1, are suspended in 150 ml of water whilst stirring. The suspension is stirred for another 30 minutes after which 7.5 g of aspartic acid are added. The pH is adjusted to 6.0 with 0.1 M hydrochloric acid solution and stirred for 48 hours, maintaining the pH at 6.0 using a pH-stat apparatus. During the reaction 385 mg of sodium cyanoborohydride is added in portions of 30-40 mg, at regular intervals. After 48 hours, an additional 200 mg of sodium cyanoborohydride is added in one portion. Once the evolution of hydrogen has ceased, the pH is raised to 7.0 and any unreacted aspartic acid and inorganic salts are removed from the reaction mixture by means of nanofiltration and the residue is lyophilised.

The product was tested for its copper(II) binding capacity, using a copper(II)-selective electrode. 100 mg of product were dissolved in 100 ml of water and titrated with a 0.4 M $CuCl_2$ solution until a residual Cu^{2+} concentration of 1×10^{-5} M had been reached. The copper-binding capacity was found to be 0.9 mmol/g.

Example 3

Reductive amination of MACS with iminodiacetic acid

1.0 g of lyophilised MACS, prepared according to Example 1, is suspended in 50 ml of water whilst stirring. The suspension is stirred for another 30 minutes after which 1.5 g (3 mmol) of iminodiacetic acid are added. The pH is adjusted to 6,0 with 0.1 M hydrochloric acid solution and stirred for 72 hours, maintaining the pH at 6.0 using a pH-stat

apparatus. During the reaction 378 mg of sodium cyanoborohydride is added in portions of 30-40 mg, at regular intervals. After 72 hours, an additional 200 mg of sodium cyanoborohydride is added in one portion. Once the evolution of hydrogen has ceased, the pH is raised to 7.0 and any unreacted iminodiacetic acid and inorganic salts are removed from the reaction mixture by means of nanofiltration and the residue is lyophilised. It was determined by nitrogen analysis that only partial reductive amination (35-40 % of the available aldehyde groups) had taken place.

The product was tested for its copper(II) binding capacity, using a copper(II)-selective electrode. 100 mg of product were dissolved in 100 ml of water and titrated with a 0.4 M $CuCl_2$ solution until a residual Cu^{2+} concentration of 1 x 10^{-5} M had been reached. The copper-binding capacity was found to be 0,7 mmol/g.

Example 4

Reductive amination of MACS with 1-octyl amine

3.0 g of lyophilised MACS, prepared according to Example 1, are suspended in a well-stirred mixture of 200 ml of water and 50 ml of ethanol. The suspension is stirred for another 30 minutes after which 1.0 g of sodium cyanoborohydride is added. The pH is adjusted to 6.0 with 0.1 M hydrochloric acid solution and 548 mg (25 mol-% with respect to the aldehyde groups MACS) of 1-octyl amine are added dropwise whilst maintaining the pH at 6.0 using a pH-stat apparatus. Stirring and pH-control are continued overnight. The pH is raised to 7.0 and any unreacted 1-octyl amine and inorganic salts are removed from the reaction mixture by means of nanofiltration and the residue is lyophilised.
The surface tension reduction of the product was measured as a function of its concentration, using a drop tensiometer. The results are shown in Table 1.

Example 5

Reductive amination of MACS with 1-dodecyl amine

3.0 g of lyophilised MACS, prepared according to Example 1, are suspended in a well-stirred mixture of 200 ml of water and 50 ml of ethanol. The suspension is stirred for another 30 minutes after which 1.0 g of sodium cyanoborohydride is added. The pH is adjusted to 6.0 with 0.1 M hydrochloric acid solution and 785 mg (25 mol-% with respect to the aldehyde groups present in MACS) of 1-dodecyl amine are added dropwise whilst maintaining the pH at 6.0 using a pH-stat apparatus. Stirring and pH-control are continued overnight. The pH is raised to 7.0 and any unreacted amine and inorganic salts are removed from the reaction mixture by means of nanofiltration and the residue is lyophilised.

The surface tension reduction of the product was measured as a function of its concentration in water, using a drop tensiometer. The results are shown in Table 1.

Table 1. Surface tensions of aqueous solutions of the products prepared according to Examples 4 and 5, as determined with a drop tensiometer.

Concentration	Surface tension (mN/m) at 25 °C	
(g/l)	Example 4 (N-octyl)	Example 5 (N-dodecyl)
0.1	70.7	68.9
0.5	61.2	57.7
1.0	52.3	52.2
2.0	48.0	47.4
5.0	43.8	38.6
10	41.9	29.4
25	40.4	30.7

A significant lowering of the surface tension was observed, the largest effect being observed for the dodecyl amino derivative, which is in agreement with theoretical predictions.

Claims

1. An oxidised carbohydrate derived from a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, the 1,2-dihydroxyethylene groups having at least partially been oxidised to dialdehyde groups, and a part of the aldehyde groups having been oxidised to carboxylic acid groups, the ratio between aldehyde groups and carboxyl groups being between 25/75 and 80/20.

2. An oxidised carbohydrate according to claim 1, containing on average 0.1-1.5 carboxyl groups and 0.5-1.9 aldehyde groups per oxidised 1,2-dihydroxyethylene group.

3. An oxidised carbohydrate according to claim 2, containing on average 0.5-1.3 carboxyl groups and 0.7-1.5 aldehyde groups per oxidised 1,2-dihydroxyethylene group.

4. An oxidised carbohydrate according to any one of claims 1-3, containing on average 0.1-1.2 carboxyl groups and 0.3-1.2 aldehyde groups per repeating unit.

5. An oxidised carbohydrate according to claim 1 or 2, wherein the carbohydrate is starch, amylose or amylopectin or a modification thereof.

6. An oxidised carbohydrate according to claim 1 or 2, wherein the carbohydrate is cellulose or a modification thereof.

7. An oxidised carbohydrate according to claim 1 or 2, wherein the carbohydrate is a 2,1-fructan.

8. A process for producing an oxidised carbohydrate according to any one of claims 1-7, comprising oxidising a dialdehyde carbohydrate obtainable by oxidising a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, the oxidation of the dialdehyde carbohydrate being performed with a catalytic amount of molecular halogen, in particular molecular bromine.

9. A process according to claim 8, wherein the oxidation with molecular halogen is performed at a pH between 3 and 7.

(78)

10. A process according to claim 7 or 8, wherein the molecular halogen is produced in situ by reaction of halide with a carboxylic peracid.

11. A process for producing an oxidised, amino-substituted carbohydrate, comprising reductively aminating residual aldehyde groups in the oxidised carbohydrate obtained by the process according to any one of claims 8-10.

12. An amino-substituted oxidation product derived from a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, these dihydroxyethylene groups having at least partially been oxidised to dialdehyde groups, the product containing on average 0.1-1.5 carboxyl groups and 0.1-1.9 substituted amine groups per oxidised 1,2-dihydroxyethylene group.

13 An amino-substituted oxidation product according to claim 12, containing on average 0.1-1.2 carboxyl groups and 0 3-1.2 substituted amino groups per repeating unit.

14. An amino-substituted oxidation product according to claim 12 or 13, wherein said substituted amino group has the formula $-NR^1R^2$, wherein R^1 represents hydrogen, a C_1-C_{20} alkyl, alkenyl or alkynyl group optionally substituted with carboxy, hydroxy, C_1-C_{12} alkoxy, amino, carbamoyl and/or aryl, including natural and synthetic amino acid residues, and R^2 represents hydrogen, amino, substituted amino, hydroxy, alkoxy, or a C_1-C_{12} alkyl, alkenyl or alkynyl group optionally substituted with carboxy, hydroxy, C_1-C_{12} alkoxy, amino and/or carbamoyl, or a substituted iminomethyl group, or R^1 and R^2, together with the nitrogen atom to which they are bound, represent a three- to seven-membered heterocyclic system, optionally containing one or more further heteroatoms selected from nitrogen, oxygen and sulphur and optionally substituted with carboxy, hydroxy, oxo, C_1-C_{12} alkyl, alkenyl, alkynyl or alkoxy, amino, carbamoyl and/or aryl

ABSTRACT

A novel oxidation product derived from a carbohydrate containing 1,2-dihydroxyethylene groups in its repeating units, can be obtained by at least partially oxidizing the 1,2-dihydroxyethylene groups of the carbohydrate to dialdehyde groups, and oxidizing a part of the aldehyde groups to carboxylic acid groups. The oxidation product has a regular structure with alternating aldehyde groups and carboxyl groups in a ratio of about 1:1. It can be further transformed to an amino-substituted oxidation product by reductive amination of at least a part of the remaining aldehyde groups.

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL DESIGN, NATIONAL STAGE OF PCT OR CIP APPLICATION)

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Carbohydrate oxidation products and derivatives

the specification of which: (complete (a), (b) or (c) for type of application)

REGULAR OR DESIGN APPLICATION

a. [ ] is attached hereto.
b. [ ] was filed on as Application
Serial No. and was amended on
(if applicable)

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. [x] was described and claimed in International application No. PCT/NL99/00673
filed on 2 November 1999
and as amended on (if any)

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a).

In compliance with this duty there is attached an information disclosure statement 37 CFR 1.97

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35. United States Code paragraph 119 of any foreign application (s) for patent of inventor's certificate listed below and have also identified below any foreign application for patent of inventor's certificate having a filing date before that of the application on which priority is claimed.

(complete (d) or (e))

d. [ ] no such applications have been filed
e. [X] such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION

Country	Application Number	Date of filing (day, month, year)	Date of Issue (day, month, year)	Priority claimed
Europe	98203706.1	2 November 1998		Yes

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION

____

____

CONTINUATION-IN-PART

(Complete this part only if this is a continuation-in-part application)

I hereby declare claim the benefit under Title 35, United States code, paragraph 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claim of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, paragraph 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

____

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

____

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric Jensen, Reg. No. 37,855, and Thomas W. PERKINS, Reg. No. 33,027 and Roland E. Long, Jr. Reg. No. 41,949 c/o YOUNG & THOMPSON, Second Floor, 745 South 23rd Street, Arlington, Virginia 22202.

Address all telephone calls to Young & Thompson at 703/521-2297.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full name of sole or first inventor: **BESEMER, Arie Cornelis**

Inventor's signature

Date 7 May 2001 Country of Citizenship: The Netherlands

Residence: AMERONGEN, The Netherlands NLX

Post Office Address: H. van de Boschstraat 111, NL-3958 CC AMERONGEN, The Netherlands

2-00 Full name of second inventor: **JETTEN, Jan Matthijs**

Inventor's signature

Date 7 May 2001 Country of Citizenship: The Netherlands

Residence: ZEIST, The Netherlands NLX

Post Office Address: Costerlaan 3b, NL-3701 JL ZEIST, The Netherlands

3-00 Full name of third inventor: **VAN DOREN, Hendrik Arend**

Inventor's signature

Date 7 May 2001 Country of Citizenship: The Netherlands

Residence: AMERSFOORT, The Netherlands NLX

Post Office Address: Prins Bernhardlaan 5, NL-3818 ZE AMERSFOORT, The Netherlands

4-00 Full name of fourth inventor: **VAN DER LUGT, Jan Pieter**

Inventor's signature

Date 7 May 2001 Country of Citizenship: The Netherlands

Residence: AMERSFOORT, The Netherlands NLX

Post Office Address: Schone van Boskoopgaarde 15, NL-3824 AA AMERSFOORT, The Netherlands